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Automated on-line gel permeation chromatography–gas chromatography for the determination of organophosphorus pesticides in olive oil

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Abstract

An on-line combination of gel permeation chromatography and gas chromatography has been designed using either a laboratory-built or a commercially available LC–GC apparatus to determine organophosphorus pesticides in olive oil. Gel permeation chromatography was used for sample pretreatment, viz. to separate the low-molecular-mass pesticides from the higher-molecular-mass fat constituents of the oil. A mixture of *n*-decane and the azeotropic mixture of ethyl acetate and cyclohexane was found to give an adequate separation between the fat and the organophosphorus pesticides. The pesticide-containing fraction, monitored by a UV detector, was transferred on-line to the gas chromatograph using a loop-type interface. *n*-Decane (6%, v/v) was added to the eluent in order to widen the application range of the transfer technique towards more volatile pesticides. After solvent evaporation through the solvent vapour exit and subsequent GC separation, the compounds were selectively detected with a thermionic or a flame photometric detector. The set-up allowed the direct analysis of oil samples after dilution in the gel permeation chromatography eluent without further sample clean-up. Detection limits were about 5 and 10 μg/kg with the thermionic and the flame photometric detector, respectively, when using an injection volume of only 30 μl of the 20-fold diluted oil. The total procedure was linear in the 0.01–10 mg/kg range for both detectors. For twenty organophosphorus pesticides, the relative standard deviations were 3–13% at the 20–60 μg/kg level.

Keywords: Food analysis; Olive oil; Sample preparation; Automation; Pesticides; Organophosphorus compounds

1. Introduction

Agrochemicals such as organophosphorus pesticides (OPPs) are extensively used for the protection of olives against several insects such as the olive fly (*Bactocera (Dacus) oleae*), the olive moth (*Prays oleae*) and the black scale (*Saissetia oleae*). Maximum residue levels have been set by the FAO/WHO Codex Committee on pesticide residues for olives and olive oil and by the European Union for olives [1]. They vary from 10 (fenchlorphos, methamidophos) to 500 μg/kg (azinphos-methyl, bromophos-ethyl, diazinon, fenitrothion, malathion/malaoxon, parathion/paraoxon); for dimethoate the level is 1 mg/kg. The class of OPPs is comprised of a large number of compounds which vary widely in polarity and molecular weight. The most polar OPPs have log K_{ow} values (K_{ow}=octanol–water distribution coefficient) lower than −2.56, which is the value for dimefox, while the strongly apolar OPPs

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have log K_{ow} values of as high as 5.95 (temefox) [2]. Their molecular masses vary from 141 (methamidophos) to 457 (dioxathion) g/mol. These widely different characteristics seriously complicate the trace-level determination of the whole range of OPPs by one method. As a result, straightforward techniques which combine a short analysis time, sufficient selectivity and adequate sensitivity are in high demand.

Because of its separation speed, its high separation power and the variety of selective detection methods that can be used such as thermionic or nitrogen–phosphorus (NPD), flame photometric (FPD) and mass spectrometric (MS) detection [3–6], the preferred method for the determination of OPPs is capillary gas chromatography (GC). The preparation of oil samples for the determination of pesticides by GC requires the complete removal of the high-molecular-mass fat from the sample to maintain the chromatographic system in working order. Clean-up can be performed on a gravity-driven normal-phase liquid chromatographic (LC) column packed with Florisil or silica [7,8], and by gel permeation chromatography (GPC) [9,10]. The coelution of part of the fatty constituents with the more polar OPPs renders the normal-phase LC procedure less suitable as a universal clean-up method. In addition, when performed in an off-line fashion, both techniques are rather time-consuming and laborious, and require large volumes of both the sample and organic solvents. Furthermore, after isolation of the analytes, the eluate often has a volume of several millilitres. Consequently, solvent evaporation (to ca. 0.5–1 ml) has to be performed and, next, a rather small aliquot of 1 to 2 μl is injected into the GC. Although these procedures are generally accepted, analyte losses due to sample manipulation will occur readily and automation can not be achieved easily.

The on-line combination of GPC and GC eliminates most of the above problems. Since the labour requirement decreases, sample throughput will be higher and, with the on-line set-up, full automation becomes possible. Besides, since the whole fraction rather than an aliquot is introduced into the GC system, analyte detectability (expressed in μg/kg of oil) will improve. This implies that the sample size can be reduced to a few milligrams, i.e. a few microlitres, only. On-line GPC–GC has recently been reported for the determination of chlorinated pesticides and polychlorinated biphenyls in fat of chicken and fish extracts [11], and for the determination of OPPs in fruit extracts [12].

In principle, two interfaces can be used for the transfer of the analyte fraction from the GPC to the GC system, the on-column and the loop-type interface. However, the maximum volume of 500–800 μl that can be handled by the on-column interface [13] makes it less suitable for GPC–GC when using conventional GPC columns. The transfer of a typical GPC fraction of 2–4 ml with this interface also requires a long retention gap, and rather careful optimization of the parameters. The alternative, i.e. the loop-type interface [14], permits the transfer of volumes up to 10 ml, and only the temperature during the transfer has to be optimized. The heart of this interface is a 10-port valve with two loops; one of these is used to store the GPC fraction and the other to add an internal standard or a wash solvent. The carrier gas supply line and the retention gap are also attached to the valve. The GPC eluent continuously flows through the loop and when the fraction of interest is in the loop, transfer is started by switching the valve. Now, the carrier gas pushes the eluent into the retention gap which is in the GC oven, and is kept at a temperature above the solvent boiling point. The pressure built up by the evaporating solvent will stop the plug of liquid from penetrating further into the retention gap. Using these fully concurrent solvent evaporation (FCSE) conditions, all solvent is evaporated during transfer into the GC system.

The main disadvantage of the loop-type interface is that it can be used only for high-boiling analytes [14]. The application range can be extended by adding a so-called cosolvent [15,16] to the main solvent that is used for GPC. The cosolvent should have a boiling point which is higher than that of the main solvent. Further, it should be miscible with the main solvent and be able to wet the retention gap. While the main solvent evaporates completely during introduction into the gas chromatograph, a layer of cosolvent remains in the retention gap and serves as a temporary stationary phase in which the volatile analytes are trapped. Optimization now is more difficult, and a long megabore retention gap is required.

This paper describes the development of an on-line GPC–GC procedure which was carried out in two stages. Firstly a semi-automated laboratory-built on-line system was set up to develop an optimization strategy. The gas chromatograph was equipped with flame ionization detection (FID) and NPD. Next, the method was transferred to a commercially available Dualchrom 3000 instrument in order to obtain a fully automated software-controlled system. In this case detection of the OPPs was carried out with FPD. The optimization of the parameters for sample treatment with GPC, for collection and transfer of the pesticide fraction, and for optimal GC–FPD analysis will be discussed below and some recommendations concerning the maintenance of the system will be given. With the complete system analytical data were determined for twenty OPPs spiked in olive oil.

2. Experimental

2.1. Chemicals and solvents

Ethyl acetate, cyclohexane, methyl-*tert.*-butyl ether (MTBE), methanol, cyclopentane, methyl acetate and (all from J.T. Baker, Deventer, Netherlands) were of analytical grade. *n*-Decane (Merck, Hohenbrunn, Germany) was of synthetic quality. All solvents except the azeotropic mixtures were used without any pretreatment. The azeotropic mixtures were first mixed in the correct proportions; next, the mixture was distilled and the fraction with the correct boiling point (71.6°C for ethyl acetate–cyclohexane; 49.3°C for methyl acetate–cyclopentane) collected. In the final stage of the research project this procedure was carried out using batches of 1 l.

Stock solutions (1 mg/ml) of the OPPs, which had a purity of 98% or better and were obtained from various suppliers, were prepared in ethyl acetate. Mixtures used for spiking and calibration were prepared by mixing these stock solutions with subsequent dilution in the azeotropic mixture.

2.2. Sample preparation

About 1 g of a vegetable oil sample was weighed into a 20-ml all-glass measuring flask. The flask was filled to the mark with the azeotropic mixture of ethyl acetate–cyclohexane which was used as GPC eluent. 30 μl of this solution were injected into the GPC system; this corresponds with 1.5 mg of the original olive oil.

2.3. Equipment

The on-line GPC–GC system was either a laboratory-built or a commercially available LC–GC instrument, the Dualchrom 3000 (Carlo Erba Instruments, Milan, Italy). The set-up in the final stage of the project is shown in Fig. 1. Both this and the earlier set-up are discussed below.

2.3.1. Laboratory-built instrument

GPC

The GPC part of the instrument consisted of a Gilson 305 pump (Villiers-le-Bel, France), an electrical six-port injection valve (MUST; Spark Holland, Emmen, Netherlands), a 300×7.5 mm I.D. column packed with PLGel with a particle size of 5 μm and 50 Å pores (Polymer Laboratories, Church Stretton, UK), and a variable-wavelength UV detector (Carlo Erba Instruments). The pump delivered an azeotropic ethyl acetate–cyclohexane mixture with 6% (v/v) *n*-decane at a flow-rate of 1 ml/min. In between runs the flow-rate was set at 50 μl/min to save solvents. The injection volume was 30 μl and UV detection was carried out at 254 nm. The fraction containing the pesticides started to elute after 7.8 min in a volume of 2.5 ml, i.e. in 2.5 min.

GPC–GC interface

The interface was built from two electrical six-port valves (MUST; Spark Holland). One of these, the so-called gas valve, was used to deliver the carrier gas, and the other, the so-called liquid valve was used to mount the sample storage loop. The carrier gas was led to the GC column either directly via a glass Y-piece to which the retention gap was connected or to the same Y-piece via the liquid valve with the 3-ml loop. To ensure that the loop was empty before being filled with the pesticide fraction, another MUST valve equipped with a nitrogen gas supply at 1 bar was connected to the liquid valve. The carrier gas supply was constructed from a pressure reducer set at 2 bar and a flow regulator

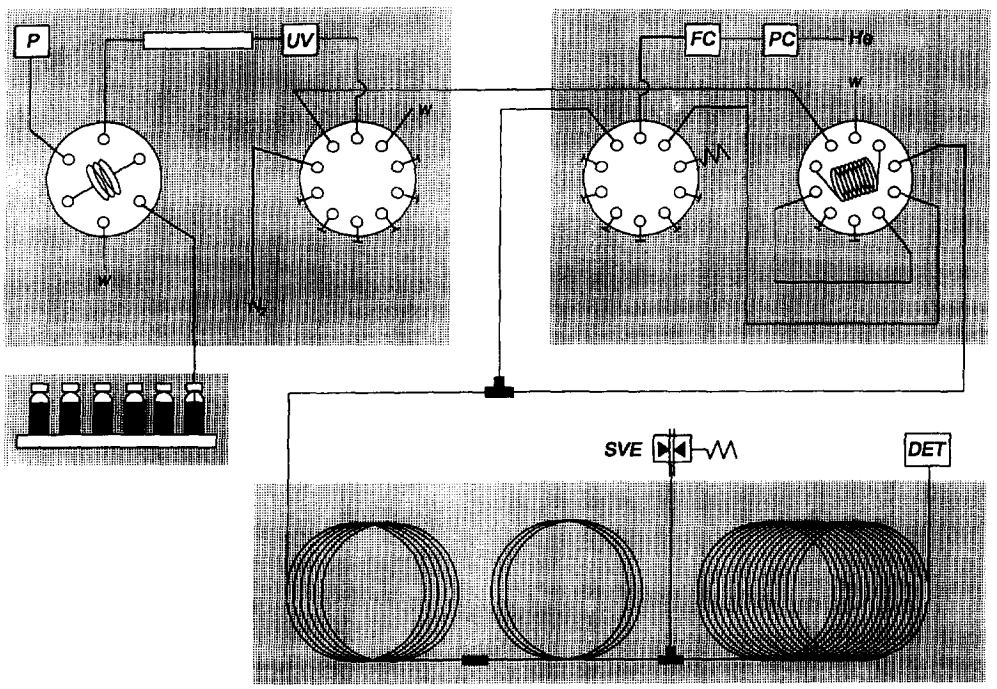

Fig. 1. Scheme of the total analytical set-up consisting of an autosampler, a GPC system for sample clean-up, a loop-type interface equipped with a 3-ml loop and a GC system with a retention gap, retaining precolumn and analytical column. P, solvent pump for GPC; UV, UV–Vis detector; FC, flow controller; PC, pressure controller; W, waste outlet; N_2, nitrogen purge gas; SVE, solvent vapour exit; DET, detector.

preset at 2 ml/min. The transfer was carried out at 105°C. The solvent vapour was released from the gas chromatograph using a three-way gas valve (Sirai, Milan, Italy) as early solvent vapour exit. This valve was kept open for 4.8 min and was closed just before the cosolvent had evaporated.

GC system

In the gas chromatograph (Model 5890 Series II; Hewlett-Packard, Palo Alto, CA, USA) equipped with FID and NPD, a 10 m×0.53 mm I.D. retention gap deactivated with diphenyltetramethyldisilazane (DPTMDS; BGB Analytik, Zürich, Switzerland) was connected at the inlet side to the glass Y-piece of the loop-type interface and at the outlet, via a press-fit connection to a 3-m section of the GC column serving as retaining precolumn. Using a press-fit Y-piece, the solvent vapour exit was installed between the retaining precolumn and the remaining part of the GC column (25 m×0.32 mm I.D. coated with 0.25-μm DB5-MS; J&W, Folsom, CA, USA). The Y-piece was connected to a three-port valve on top of the gas chromatograph via a 20 cm×0.32 mm I.D. fused-silica capillary. In the "closed" position of the vapour exit, the valve was connected to a 2 m×75 μm i.d. fused-silica resistance, which tolerated a purge flow of about 1% of the column flow. The exit was programmed to switch to the closed position just before the cosolvent had evaporated. The column temperature during transfer was 105°C. After closing of the solvent vapour exit, the temperature programme was started at 5°C/min to 240°C, followed by a second gradient to 300°C at 10°C/min and a final hold of 5 min at 300°C.

2.3.2. Dualchrom 300

With the Dualchrom 3000, the GPC–GC system was built using the connecting capillaries, the GPC columns, UV–Vis detector and both the injection loop and the fraction storage loop from the earlier system (cf. Fig. 1). Several parts such as switching valves, were different, but their functions were the same as described above. The nitrogen purge valve and the loop-type interface were manufactured from

Valco ten-port valves. The GPC solvent delivery pump was now a Phoenix 30 (Carlo Erba Instruments) syringe pump. The solvent vapour exit was a heated three-port gas valve, and the GC detector was an FPD80 (Carlo Erba Instruments).

Ethyl acetate–cyclohexane was again the GPC eluent at a flow-rate of 1 ml/min. The transfer conditions were optimized according to the strategy developed with the laboratory-built equipment. The retention gap, retaining precolumn and analytical column were the same as those used with the earlier procedure. The column temperature programme was 105°C during transfer and evaporation of the main part of the solvent (12.17 min), and was then as reported above.

3. Results and discussion

3.1. Optimization of gel permeation chromatography

3.1.1. Eluent composition

For GPC–GC, the GPC parameters should be optimized in such a way that the fraction of interest is separated from the major part of the other sample constituents which mainly consists of triglycerides. Several parameters were considered with regard to the eluent composition for GPC sample treatment. A rather polar solvent should be one of the constituents of the solvent mixture in order to minimize interaction between the analytes and the support of the GPC column. The solvent mixture should be UV-transparent, which simplifies optimization of the GPC separation using a UV–Vis detector. Its boiling point should be as low as possible, because then the widest application range is obtained after transfer of the relevant fraction to the GC system. Several solvents such as, e.g., tetrahydrofuran and dichloromethane, were excluded because of safety, health and/or environmental disposal reasons or because of their incompatibility with the detectors used. Therefore, *n*-alkanes and cyclic alkanes with added MTBE, methanol or ethyl acetate were used to study the separation between the triglycerides and the OPPs. Olive oil spiked with OPPs at a level of 100–500 mg/kg was used in this part of the study, which was further diluted in GPC eluent in order to obtain 10% (v/v) of oil.

Initially *n*-pentane without a polar modifier was used as eluent for the GPC preseparation on a PLGel column with a pore size of 50 Å. OPPs were added to olive oil which was diluted in the eluent. Many OPPs had additional retention on the PLGel column. The last eluting OPP, azinphos-methyl, eluted after about 3 h (183 ml). The addition of up to 95% (v/v) of MTBE resulted in reduced chromatographic retention, but the OPPs still eluted in a volume larger than twice the void volume of the GPC column. When using methanol instead of MTBE as modifier, similar behaviour of the fat and OPPs was observed. Even the use of pure MTBE or methanol did not give the desired result.

The best results were obtained when using a cyclic alkane such as cyclohexane with methyl or ethyl acetate as modifier. As could be expected with such eluents, the separation between the fractions containing the OPPs and the fat became worse at higher concentrations of the polar modifier. Fig. 2B which illustrates the change in volume of the OPP-containing fraction at different eluent compositions, shows that the volume of this fraction decreases rather rapidly when going from 15 to about 45% (v/v) of ethyl acetate. The volume should not become too large, since then transfer to the GC system will become difficult. At higher percentages of the modifier the volume remains essentially constant. On the other hand the fat should be completely separated from the OPP fraction. Fig. 2A demonstrates that at 75% (v/v) of ethyl acetate or more the fractions start to coelute. A compromise between fraction size and complete separation was found by selecting the azeotropic composition of ethyl acetate–cyclohexane. The beneficial effect of using the azeotropic mixture was the very high reproducibility of eluent production, which resulted in very stable retention times in GPC.

Similar results were obtained for mixtures of cyclopentane and methyl acetate, which have the more advantageous boiling point range of 43–49°C. With this mixture at compositions of lower than 60% (v/v) of methyl acetate the pesticides were fully separated from the fat. With methyl acetate–cyclopentane (70:30, v/v) some of the pesticides coeluted with the fat peak. Furthermore, the fraction

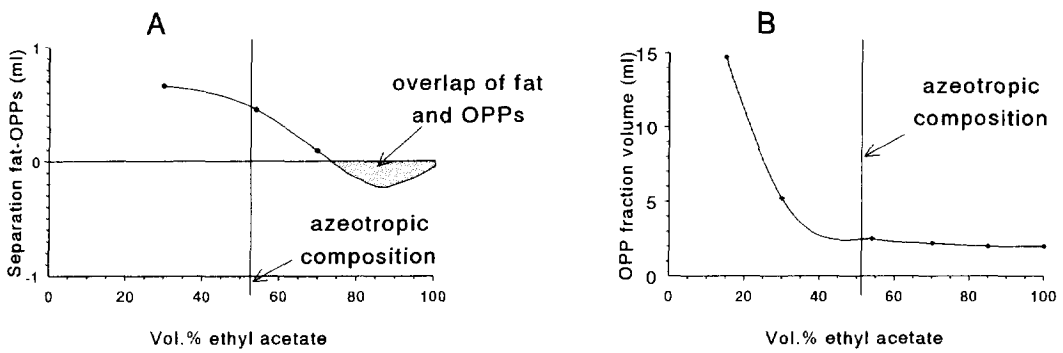

Fig. 2. Influence of the eluent composition of ethyl acetate–cyclohexane on (A) the separation between the fat and OPP fractions and (B) the volume of the OPP-containing fraction. GPC column: 30 cm×7.5 mm I.D. PLGel, 50 Å, 5 μm; sample: 50 g/l olive oil in eluent; spiking level: 100–500 mg/kg; injection volume: 30 μl.

size increased from 1.5 ml for methyl acetate–cyclopentane 50:50 (v/v) to more than 5 ml for the 10:90 (v/v) mixture. Since cyclopentane is rather expensive compared with cyclohexane, it was decided to use the azeotropic mixture of ethyl acetate and cyclohexane for all further work, although the larger volume had then to be transferred.

3.1.2. GPC stationary phase

Further studies were carried out with the ethyl acetate–cyclohexane azeotrope in order to reduce the volume of the OPP-containing fraction. In addition to the stationary phase used above, two other GPC columns were tested using the same spiked oil (100–500 mg/kg). As a reference point, the separation obtained with the 50 Å pore size column is shown in Fig. 3A. The volume of the OPP-containing fraction is about 2.5 ml. However, when the same stationary phase was used with a pore size of 500 Å (Fig. 3B), the fraction volume was reduced to 2 ml. It has to be added that the first OPP now eluted on the tail of the fat fraction which would make the GPC-to-GC transfer rather difficult. The most adequate way of reducing the peak volume of the OPP fraction is obviously the use of a smaller-bore column. For the PLGel phase, however, no such commercial column was available at the time. Therefore, a column with an internal diameter of 3 mm packed with 100 Å styrene–divinylbenzene copolymer was used instead (Polymer Standard Service; 5 μm d_p, 300×3 mm I.D.). As can be seen from Fig. 3C, the volume of the OPP fraction was indeed reduced to about 0.3 ml, but the baseline separation between fat and the fraction of interest could not be restored. It was therefore decided to use the 7.5-mm I.D. PLGel 50 Å column in all further experiments.

3.1.3. Guard column

During optimization of the transfer it became clear that some of the fat, which eluted from the GPC column with a rather long tail (not visible on a UV–Vis detector trace), was transferred as well. The transfer of part of the fat resulted in loss of detector response and in deteriorated peak shapes in the chromatogram after ca. 50 analyses. The fat also became visible as it settled in the retention gap where it formed rather thick droplets. We tried to retain the tail of the fat fraction prior to its entrance

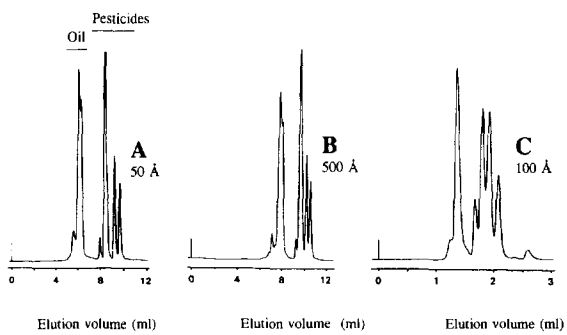

Fig. 3. Separation of OPPs and fat by means of GPC using ethyl acetate–cyclohexane azeotrope as eluent with a (A) 300×7.5 mm I.D. PLGel 5 μm, 50 Å; (B) 300×7.5 mm I.D. PLGel, 5 μm, 500 Å; (C) 300×3 mm I.D. PSS SDV, 5 μm, 100 Å. Flow-rate, 1 ml/min (A, B) or 0.4 ml/min (C).

into the storage loop of the transfer interface by means of a 10×2 mm I.D. guard column packed with silica or alumina. After each run this guard column was cleaned with pure ethyl acetate. The insertion of the guard column between valves 2 and 3 (cf. Fig. 1) did not influence the collection of the OPP-containing fraction. However, the expected fat retention did not occur; after some 50 analyses the droplets of fat again became visible in the retention gap. Consequently, it is recommended to exchange the retention gap after 50 runs.

3.2. Optimization of transfer

3.2.1. General optimization

The temperature during a loop-type transfer has to be chosen such that the flow of liquid into the GC system is stopped by the partial pressure of the solvent. This means that the temperature has to be above the boiling point. Important aspects regarding optimization of the minimum temperature therefore are the solvent boiling point and the pressure in the interface. The solvent boiling point can be calculated from the pressure of the helium gas supply that has to be used in the interface. Under optimized conditions this pressure is 1.5–2 bar. For most solvents the boiling point can be calculated by adding 10°C to the standard boiling point per bar of gas pressure used in the interface. When a low pressure (1 bar or less) is used in the interface, the transfer of large fractions will require a very long time, which can be in the order of 10–20 min. Another disadvantage of a low pressure is that pressurizing the GC column after closing of the solvent vapour exit will take rather a long time. At a high pressure (2.5–4 bar) the transfer time is reduced to a few minutes. However, when using such a high pressure, the liquid will be pushed rather far into the retention gap. This can only be compensated for by a high transfer temperature which, in its turn, narrows the application range of the method. It was therefore decided to use a pressure of 2 bar.

Minimum temperature during transfer

With the loop-type interface, the most important parameter for correct solvent transfer is the transfer temperature. The minimum oven temperatures for eluent transfer under FCSE with the loop-type interface which were experimentally determined by Grob and Läubli [17] can be used as a first approximation during optimization. Initially, we evaluated the minimum transfer temperatures of several solvents and solvent mixtures for a 10-m retention gap by transferring 500-μl fractions of solvent containing 10–20 ng of *n*-alkanes. With a 10-m retention gap, the minimum transfer temperature for most solvents was 21–24°C above the standard boiling point. The application range, expressed in elution temperatures of *n*-alkanes, was limited to those that eluted at temperatures 120–130°C above the transfer temperature. For the solvent mixture used for GPC in the current study, a transfer temperature of 95°C was the minimum temperature that could be used. This resulted in an application range expressed in carbon numbers of the *n*-alkanes of C_{21} and higher.

3.2.2. Addition of cosolvent

The addition of a higher boiling cosolvent [16] to the GPC eluent had to be considered, because use of the loop-type interface will restrict the application to high-boiling analytes, i.e. will exclude the volatile OPPs. The main solvent evaporates completely during transfer, but part of the higher-boiling cosolvent remains on the inner wall of the retention gap and acts as a temporary stationary phase for volatile compounds. It can already be stated here that the addition of *n*-decane, which was used as cosolvent in this study, to the eluent did not influence the GPC separation of the fat and pesticide fractions.

The transfer temperature has to be optimized such that evaporation occurs in the very first part of the retention gap. The use of a glass window in the GC oven door is thus very beneficial, because it allows the optimization of the evaporation site by visual inspection. The temperature can now be decreased/increased such that the position of this site is about 0.5 m from the entrance of the retention gap. Also, one should keep in mind that the remaining cosolvent must create a film of solvent on the retention gap wall. For the application which will be described below, a length of 10 m is required in order to have a large surface to enable the cosolvent effect. After the proper cosolvent has been selected, the only optimization left is to determine the amount that is needed to trap volatile compounds.

Initially, *n*-heptane and *n*-octane were used as

cosolvent. However, the addition of more than 10% (v/v) of these alkanes to the azeotropic mixture of ethyl acetate and cyclohexane began to influence the volume of the OPP-containing fraction. Furthermore, even the addition of more than 20% (v/v) of *n*-octane did not create the expected cosolvent effect. The use of *n*-decane as cosolvent gave the desired result. Up to 10% (v/v) of decane could be used without too much change in the GPC separation. Depending on the temperature used, the addition of 2 to 6% (v/v) of *n*-decane resulted in the required effect (see below).

It was found that the minimum temperature needed in order to restrict the evaporation of the azeotropic mixture of ethyl acetate–cyclohexane to the first 0.5-m section of the 10 m×0.53 mm I.D. retention gap was 33°C above the standard boiling point, that is, a temperature of 10°C above the optimized minimum temperature for standard loop-type transfer, in which the evaporation site is in the last part of the retention gap. Addition of the cosolvent resulted in an extended application range, viz. C_{13}–C_{30}. As an illustration, Fig. 4 shows the result of transferring *n*-alkane fractions in the absence and presence of cosolvent. The temperatures used were 95 and 105°C, respectively.

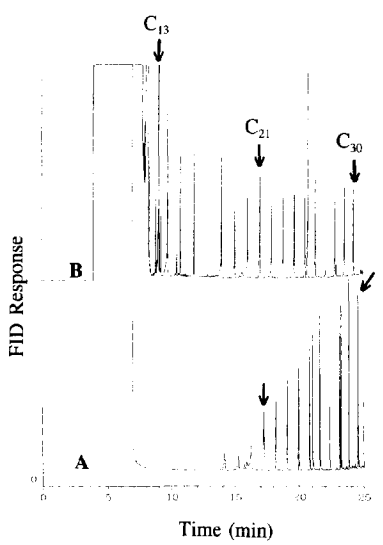

Fig. 4. GC–FID chromatograms of 500 μl of a standard *n*-alkane mixture in ethyl acetate (A) without addition of *n*-decane, and transferred at a temperature of 95°C (application range: C_{21}–C_{30}), and (B) with addition of 6% (v/v) of *n*-decane and transferred at 105°C (application range: C_{13}–C_{30}). All alkanes produce proper peak shapes.

Solvent vapour exit and solvent elimination time

Under conventional operating conditions, i.e. in the absence of cosolvent, the point in time at which the solvent vapour exit had to be closed was not very critical. No compounds that were quantitatively retained in the retention gap during the actual transfer into the GC system were lost, not even when the solvent vapour exit was kept open for up to 2 min after the transfer had been completed (indicated by a rapid pressure drop in the interface). With the cosolvent trapping technique, the time span for which the solvent vapour exit was open became very important. Once the cosolvent had evaporated, the volatile analytes were no longer retained, and started to elute via the exit. The time delay between the end of the transfer (pressure drop) and the end of the cosolvent evaporation process (loss of volatiles) depends on the percentage and nature of the cosolvent. For practical use of the cosolvent technique, an additional time of 20–60 s could be used. For 6% (v/v) of *n*-decane in the azeotropic mixture of ethyl acetate and cyclohexane, the optimum delay time was 30 s. With shorter delay times, a large solvent peak was observed in the chromatogram. With longer delays, the volatile compounds started to be lost via the solvent vapour exit.

Volume of eluent fraction

For a very large eluent fraction, as was used in the present GPC–GC study (3 ml; cf. above), the temperature optimization for conventional loop-type interface conditions (no cosolvent) gave a minimum temperature that was some 5°C higher than for the 500-μl fractions. The initial temperature of 105°C resulted in broad peaks for C_{20} and C_{21} caused by "band broadening in time". The 5°C higher temperature reduced the transfer time, which kept band broadening in time minimal. On the other hand it also caused the loss of the most volatile alkane in the application range, i.e. C_{21}. When a cosolvent was added to the eluent, this effect was not observed (transfer at 105°C), because the analyte is trapped in a solvent film rather than by cold internal trapping. A note of warning has to be added. One can not

increase the volume of the fraction indefinitely, because the cosolvent film then becomes too long and starts flooding the retaining precolumn.

On-line GPC–GC

As a first approach, the total GPC–GC system was used with an FID or NPD as detector. Fig. 5 shows typical chromatograms obtained after on-line GPC–GC of 30 μl of a 5% (v/v) solution of a spiked olive oil in the GPC eluent with FID or NPD. Almost all compounds show good peak shapes. Further use of FID was omitted because it was neither sensitive nor selective enough. The detection limits for the GC–FID analysis were in the high μg/kg range, while they were close to 1 μg/kg when using NPD. Except for mevinphos, acephate and monocrotophos, which are either too volatile or too polar to be analyzed with the present system, the recoveries were in the range of 80–105% at the 20–60 μg/kg level, which is satisfactory for such a complicated analysis. Because fenamiphos and disulfoton coeluted, the sum of the recoveries was used for these two analytes. The high recovery of carbophenthion is probably due to an interfering compound present in the spiking solution, because there was no peak in the unspiked sample. Relative standard deviations (R.S.D.s) at the 20–60 μg/kg level, measured over a period of 3 days and determined by repeated analysis of a spiked sample, were 4–10% (n=9), except for carbophenthion (22%). Data on recovery, R.S.D. and linearity for the individual OPPs are listed in Table 1. Although these data suggest that the system was very stable, it occasionally happened that the sensitivity of the NPD bead decreased rather rapidly. In such a case, it was not possible to restore the system sensitivity again unless the bead was replaced.

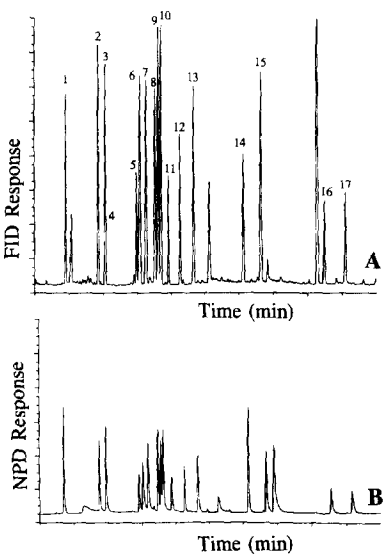

Fig. 5. Chromatograms of a spiked olive oil sample obtained after on-line GPC–GC with (A) FID and (B) NPD detection. Spiking level, (A) 2–6 mg/kg and (B) 20–60 μg/kg; transfer volume, 3 ml (ethyl acetate–cyclohexane with 6%, v/v, of *n*-decane); transfer temperature, 105°C. Peak assignment as in Table 1.

3.2.3. Fully automated GPC–GC

In the final stage of the project the system was rebuilt using a Dualchrom LC–GC system, which allowed software-controlled analysis of oil samples. An FPD system was installed instead of the NPD system, since experience with large-volume injections in our laboratory showed that this detector is very stable over a long period of time. With the complete set-up of GPC–UV–loop-type transfer-GC, olive oil samples with and without spiking at the trace-level were analyzed. Fig. 6 shows a typical chromatogram obtained after on-line GPC–GC–FPD of 50 μl of a 5% (v/v) solution of olive oil in cyclohexane. Again, almost all compounds show good peak shapes. For mevinphos, acephate and monocrotophos, the same problems were observed as have been mentioned above. The recoveries for the other test OPPs were in the range of 70–130% at the 20–60 μg/kg level, which agrees rather well with the previous data. The R.S.D. values for ten subsequent analyses of the same sample spiked at the 20–60 μg/kg level were 3–13%.

The linearity for the total procedure was also tested in the 10–10 000 μg/kg range with the new system. As was expected from our earlier experience with FPD, the detector showed good linearity in this range. Per run, 30 μl of a 5 vol.% oil solution were injected, which means that actually only 1.5 mg of oil was analyzed; or, in other words, between 15 pg and 15 ng of an analyte was injected into the system. Table 2 shows data on R.S.D. values and regression coefficients for the individual OPPs tested. Detection limits of the analytes in real oil samples were in the 1–5 μg/kg range.

Table 1
Recovery and R.S.D. values for OPPs at the 20–60 μg/kg level ($n=10$), and linearity data of log concentration vs. log peak area plot in the 0.01–10 mg/kg range

Compound	Recovery (%)	R.S.D. (%)	$y=ax+b$		R^2
			a	b	
1 Sulphotep	96	6	0.93	5.0	0.998
2 Diazinon	100	6	0.86	4.7	0.994
3 Fenamiphos[a]	–	–	–	–	–
4 Disulphoton[a]	100	4	–	–	–
5 Fenchlorphos	100	8	0.98	4.5	0.998
6 Paraoxon-ethyl	103	7	0.93	4.4	0.991
7 Fenitrothion	97	6	0.96	4.7	0.997
8 Chlorpyriphos-ethyl	95	7	0.98	4.6	0.999
9 Fenthion[b]	100	5	0.97	4.6	0.998
10 Parathion-ethyl[b]	97	5	1.02	4.9	0.999
11 Bromophos-methyl[b]	96	4	1.06	4.6	0.998
12 Isofenphos	93	6	0.97	4.8	0.998
13 Bromophos-ethyl	83	7	0.78	5.0	0.971
14 Ethion	96	4	0.84	5.0	0.992
15 Carbophenthion[b]	162	22	0.80	4.8	0.993
16 Azinphos-methyl[b]	92	10	0.87	4.8	0.992
17 Azinphos-ethyl	95	7	0.93	4.9	0.999

Nine data paints for on-line GPC–GC–NPD of spiked oil samples.
[a] Compounds co-elute; overall recovery and R.S.D. value given.
[b] OPPs applied to olive crops.

3.2.4. Maintenance of the system

Exchange of retention gap

As was pointed out above, even if GPC pretreatment is performed, some of the fat is transferred to the GC system. This has no consequences for the performance of the GC system. However, after about 50 analyses the amount of fat present in the system is so large that the peaks start to deteriorate and analyte detectability begins to decrease seriously. Replacement of both the retention gap and the retaining precolumn solved the problem. It is advisable to use new capillaries rather than cleaning the old ones, because cleaning and testing takes quite some time, and it is not always possible to restore the initial system performance. For unknown reasons, the installation of several retention gaps from various suppliers appeared to be unsuccessful. Either unacceptable peak tailing occurred or difficulties during the GPC-to-GC transfer were observed.

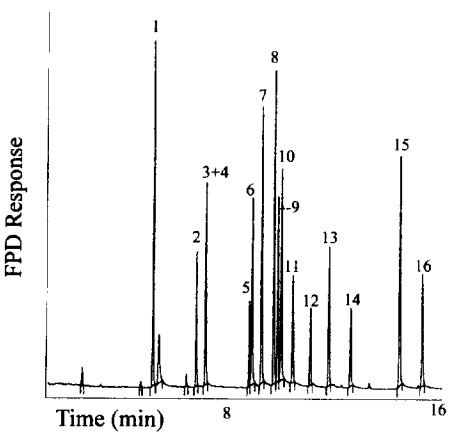

Fig. 6. GC–FPD chromatogram of olive oil sample spiked at the 20–60 μg/kg level with twenty OPPs after on-line GPC sample treatment. 3 ml of the eluate (ethyl acetate–cyclohexane with 6%, v/v, of *n*-decane) were transferred via the loop-type interface at 105°C. No detectable levels of the spiked analytes were detected in the blank sample. Peak assignment as in Table 1.

GC detector

During the present study, it became clear that FPD is better suited for the detection of P-containing compounds than NPD. The sensitivity of both detectors is essentially the same nowadays, but with NPD, sensitivity changes with time due to degradation of the bead in the detector, even under standard GC operating conditions. The large solvent peak (in this case an ethyl acetate-containing solvent) that is

Table 2
Repeatability at the 20–60 μg/kg spiking level ($n=10$) and linear regression coefficients of the analyte concentration–peak area plot (nine data points) in the 10–10 000 μg/kg range of the on-line GPC–GC–FPD for several organophosphorus pesticides

Compound	R.S.D. (%)	R^2	Compound	R.S.D. (%)	R^2
1 Sulphotep	9	0.998	10 Parathion-ethyl	3	0.999
2 Diazinon	13	0.994	11 Bromophos-methyl	8	0.998
3 Fenamiphos[a]	–	–	12 Isofenphos	8	0.998
4 Disulphoton[a]	7	–	13 Bromophos-ethyl	9	0.974
5 Fenchlorphos	4	0.997	14 Ethion	8	0.993
6 Paraoxon-ethyl	8	0.990	15 Carbophenthion	10	0.989
7 Fenitrothion	6	0.998	16 Azinphos-methyl	5	0.991
8 Chlorpyriphos-ethyl	8	0.999	17 Azinphos-etnyl	10	0.999
9 Fenthion	8	0.997			

[a] Compounds co-elute; overall recovery value given.

transferred speeded up this process. When using FPD no such problems occurred.

4. Conclusions

In the present paper, the use of an on-line GPC–GC system with various detectors is reported. Compared with conventional LC–GC systems, the volume of the fraction of interest eluted from the GPC column is fairly large, i.e. about 3 ml. In order to determine rather volatile as well as non-volatile OPPs, addition of 6% (v/v) of *n*-decane to an azeotropic mixture of ethyl acetate and cyclohexane in combination with a 10 m×0.53 mm I.D. retention gap was necessary.

The present method allows the trace-level determination of 17 OPPs of different polarities in olive oil samples. Sample preparation is essentially negligible: the sample was merely diluted with the eluent used for GPC to prevent overloading of the GPC column with triglycerides. With FPD, the sensitivity as well as the selectivity of the method were excellent. With an injection volume of only 25–50 μl of a 20-fold diluted olive oil (1–2 mg of oil injected), detection limits are at the low μg/kg level.

Although the method is promising, it appeared that it is not yet robust enough for routine analysis in industrial laboratories. The need for the exchange of retention gaps after 50 oil analyses, and the necessity for the cosolvent technique remain very critical parts of the current method. Current GPC–GC research is directed towards the miniaturization of the GPC part of the system in order to reduce the volume of the fraction that has to be transferred and, thus, the amount of fat that is "co-transferred" into the GC system.

Acknowledgments

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References

[1] C. Lentza-Rizos and E.J. Avramides, Rev. Environ. Contam. Toxic., 141 (1995) 111.
[2] D. Barceló, C. Porte, J. Cid and J. Albaigés, Int. J. Environ. Anal. Chem., 38 (1990) 199.
[3] J. Mañes, J.C. Moltó, C. Igualada and G. Font, J. Chromatogr., 472 (1990) 365.
[4] K.W. Edgell, E. Jenkins, V. López-Avila and J.E. Longbottom, J. Assoc. Off. Anal. Chem., 74 (1991) 295.
[5] J.C. Moltó, Y. Picó, G. Font and J. Mañes, J. Chromatogr., 555 (1991) 137.
[6] P.J.M. Kwakman, J.J. Vreuls, R.T. Ghijsen and U.A.Th. Brinkman, Chromatographia, 34 (1992) 41.
[7] W. Specht and M. Tillkes, Fresenius' Z. Anal. Chem., 301 (1980) 300.
[8] W. Specht and M. Tillkes, Fresenius' Z. Anal. Chem., 322 (1985) 443.
[9] K.R. Griffitt and J.C. Craun, J. Assoc. Off. Anal. Chem., 57 (1974) 168.
[10] J.F. Lawrence, Int. J. Environ. Anal. Chem., 29 (1987) 289.
[11] K. Grob and I. Kälin, J. Agric. Food Chem., 39 (1991) 1950.
[12] M. De Paoli, M.T. Barbina, R. Mondini, A. Pezzoni, A. Valentino and K. Grob, J. Chromatogr., 626 (1992) 145.
[13] K. Grob Jr., D. Fröhlich, B. Schilling, H.-P. Neukom and P. Nägeli, J. Chromatogr., 295 (1984) 55.

[14] K. Grob Jr. and J.-M. Stoll, J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986) 518.
[15] K. Grob Jr. and E. Müller, J. High Resolut. Chromatogr. Chromatogr. Commun., 11 (1988) 388.
[16] K. Grob Jr. and E. Müller, J. High Resolut. Chromatogr. Chromatogr. Commun., 11 (1988) 560.
[17] K. Grob and Th. Läubli, J. High Resolut. Chromatogr. Chromatogr. Commun., 10 (1987) 435.